J. exp. Biol. **108**, 231–245 (1984)
Printed in Great Britain © The Company of Biologists Limited 1984

AERIAL *VERSUS* AQUATIC OXYGEN CONSUMPTION IN LARVAE OF THE CLAWED FROG, *XENOPUS LAEVIS*

BY MARTIN E. FEDER

Department of Anatomy and Committee on Evolutionary Biology, The University of Chicago, 1025 East 57th Street, Chicago, Illinois 60637, U.S.A.

AND RICHARD J. WASSERSUG

Department of Anatomy, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Accepted 3 August 1983

SUMMARY

Tadpoles of *Xenopus laevis* Daudin can extract oxygen from both air and water. When these larvae have access to air, aerial oxygen uptake averages 16·6% of total oxygen consumption in normoxic water, and increases to 100% of net oxygen consumption in hypoxic water. Neither anaerobiosis nor increased buccopharyngeal ventilation occur in response to hypoxia. If tadpoles are prevented from surfacing to breathe air, they can maintain normal oxygen consumption through aquatic respiration alone in normoxic water, but not in hypoxic water. Unlike air-breathing larvae, exclusively water-breathing larvae respond to aquatic hypoxia by increasing their buccal pumping rate and by accumulating lactate. Even though *Xenopus* larvae can survive without air for many days, aerial respiration is necessary for other functions: tolerance of hypoxia, normal feeding, locomotion and buoyancy regulation.

INTRODUCTION

The need for oxygen is commonly the primary factor underlying air-breathing in aquatic vertebrates that can breathe both water and air. However, many factors other than oxygen supply *per se* may be associated with air-breathing. For example, regulation of buoyancy, hearing, nest-building, predator avoidance, body size and feeding may all bear upon air-breathing, but are partially or wholly unrelated to its respiratory function. These alternative considerations may affect the partitioning of gas exchange between air and water or may necessitate air-breathing even when the aquatic gas exchangers are adequate to supply needed oxygen.

We have examined interactions between the respiratory and non-respiratory aspects of air-breathing in tadpoles of the clawed frog, *Xenopus laevis*. *Xenopus* larvae have long been known to breathe air (Bles, 1905). Inspired air makes these tadpoles

Key words: Aerial respiration, hypoxia, tadpole.

buoyant (Gradwell, 1971; Wassersug & Feder, 1983). They almost always hover midwater with the head tipped down and the tail elevated, and scull with the tail filament to maintain their position (van Bergeijk, 1959; Gradwell, 1971). Air-breathing (and the attendant buoyancy) may have several advantages for *Xenopus* larvae. These larvae lack true gill filaments. Their large gill filters and the remainder of their buccopharynx are well vascularized for gas exchange, but these surfaces must also function in food entrapment (Wassersug, 1972; Gradwell, 1975). Air-breathing may therefore allow simultaneous feeding and respiration (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation). In tadpoles of other species (West & Burggren, 1982; Feder, 1983*a*), air-breathing is essential in compensating for aquatic hypoxia. Non-buoyant *Xenopus* larvae rest on the bottom, where their gill filters may clog (unpublished data). Air-breathing has disadvantages as well; it may make *Xenopus* larvae excessively buoyant when swimming (Wassersug & Feder, 1983) and may increase their susceptibility to predation (Feder, 1983*b*; Kramer, Manley & Burgeois, 1983).

Previous studies have examined the interactions between air-breathing and locomotor stamina (Wassersug & Feder, 1983) and between air-breathing and food uptake (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation). The present study extends this approach by examining air-breathing in response to aquatic hypoxia. Here we ask: (1) what are the physiological and behavioural responses of *Xenopus* larvae to hypoxia? (2) Are there alternative responses to aquatic hypoxia (e.g. increases in aquatic gas exchange, anaerobiosis) that are not used by larvae? (3) Are there non-respiratory functions (e.g. buoyancy regulation, feeding and swimming) that may necessitate air-breathing even when the aquatic gas exchangers alone are adequate to supply needed oxygen?

MATERIALS AND METHODS

Many of the procedures of the present study have been described previously (Feder, 1982, 1983*a*). Therefore, methods are given in detail here only if different from those of the previous studies.

Xenopus larvae were reared from laboratory stocks, and were maintained at a constant temperature, 25 °C, on a constant photoperiod (L: D 14: 10 centred at 13.00 local time) for at least 1 week before experimentation. Larvae were fed commercial baker's yeast. All experimentation was at 25 °C. Larvae were between Gosner (1960) developmental stages 25–42. All larvae were free-swimming, but the forelimbs either had not emerged or were small. Other details of care and feeding were as described by Feder (1983*a*).

Simultaneous measurements of aerial, aquatic and total rates of O_2 consumption ($\dot{V}_{O_2}$) were made in a 281-ml respirometer in which animals had access to air; see Feder (1983*a*). Measurements of the $\dot{V}_{O_2}$ in exclusively water-breathing larvae were made similarly in 60- to 120-ml respirometers without an air space. To adjust for differences in $\dot{V}_{O_2}$ due to body size, the raw $\dot{V}_{O_2}$ of each tadpole ($\mu l\ O_2\ h^{-1}$ STPD) was expressed as a percentage of $2{\cdot}9\ M^{0{\cdot}862}\ \mu l\ h^{-1}$, which is the routine $\dot{V}_{O_2}$ expected for larvae of body size M (mg dry mass) at 25 °C (Feder, 1982).

The effect of aquatic P_{O_2} on lung ventilatory frequency (f_L) was measured as

described previously (Feder, 1983*a*). The gill ventilatory frequency (f_G) was also determined for these same larvae by recording the number of buccal pump strokes during a timed interval. We use 'gill' and 'f_G' here to represent the respiratory function of the entire buccopharynx. The f_G and the f_H (heart rate) were determined visually for an additional 6–7 larvae at each of several aquatic P_{O_2} values in a 20 cm × 20 cm screen cylinder in which tadpoles had access to air (Feder, 1983*a*). The beating of the heart can be seen through the body wall of *Xenopus* larvae. The f_G of exclusively water-breathing larvae at each of several values of P_{O_2} was determined for animals confined in screen-covered plastic cubes, 37 ml volume, positioned below the water's surface so that the larvae had no access to air.

The buccal stroke volume was assessed indirectly by videotaping larvae held in screen containers (8 cm × 8 cm × 3 cm) with access to air. Animals were videotaped in full profile at each of several aquatic P_{O_2} values. The distance between the top of the head and the bottom of the buccal floor was measured from the video images when the buccal floor was maximally depressed (A) and when fully elevated (B). Percentage buccal floor depression was calculated as $100\% \times (A-B)/B$. This percentage was calculated for 5–10 pump strokes at each aquatic P_{O_2} value. Buccal stroke volume is directly proportional to this measure (Seale & Wassersug, 1979).

The lung volume of *Xenopus* larvae was measured by the methods of Scholander, Claff, Teng & Walters (1951) and Gee (1968). Immediately after an air breath, animals were killed and transferred to acid citrate (Scholander *et al.* 1951). The lung contents were released into an inverted funnel and transferred to a capillary tube of known diameter. Lung volume was calculated from the length of the gas bubble in the capillary.

Whole-body lactate concentrations of air-breathing larvae exposed to aquatic hypoxia for 1 h were determined as described by Feder (1983*a*). In a related experiment, air-breathing larvae were kept in screen cylinders (300 ml) in normoxic water for 4 h, and then the cylinders were transferred to water at either 150, 96, 84, 66 or 34 Torr. Animals transferred to 150 Torr were analysed for lactate immediately, and the others after 4 h at the experimental P_{O_2}. Lactate concentrations of exclusively water-breathing larvae were determined by holding larvae in screen cylinders (300 ml) submerged in normoxic water for 4 h, lowering the P_{O_2}, and analysing groups of larvae for lactate at regular intervals. The exact schedule of the sampling is given in Table 1 (bottom). Another group of larvae was submerged similarly in normoxic water overnight, and analysed for lactate 14 h later.

Tolerance to hypoxia was determined by holding larvae in screen cylinders (300 ml) submerged in normoxic water for 4 h, and then reducing the P_{O_2} gradually. The time and P_{O_2} value at which each tadpole became unable to swim when prodded (the 'Critical Activity Point') was recorded.

To examine the influence of air-breathing on buoyancy and the normal hovering posture of larvae, 17 larvae were placed in water in 300 ml cylinders that included an air space. Under such circumstances larvae hover midwater in a characteristic posture (see Introduction). After each tadpole was observed to breathe air, the air in its cylinder was replaced with water and the time noted until the tadpole was unable to maintain the normal hovering posture.

To examine long-term survival, 12 tadpoles of equivalent body sizes and developmental stages were chosen and held in 1000 ml flasks for 2 weeks. In six flasks the water

was normoxic but air was excluded; in the remainder the aquatic P_{O_2} was 11–26 Torr but tadpoles could breathe air.

Statistical techniques were as reported previously (Feder, 1983*a*).

RESULTS

Larvae with access to air

In normoxic water, *Xenopus* larvae breathed air regularly (Fig. 1). The f_L averaged $1{\cdot}9\,h^{-1}$ (S.D. = 1·0), and ranged from $0{\cdot}7$–$5{\cdot}3\,h^{-1}$. Air-breathing accounted for 16·6% (S.E. = 1·1%) of the total $\dot{V}_{O_2}$ at aquatic $P_{O_2} > 100$ Torr (Fig. 2). Lung volume was related to body size by the equation: lung volume (μl BTPS) = 1·8 M (dry mass, in mg) + 17·3 ($r = 0{\cdot}904$; $N = 32$). The larvae also ventilated their buccopharynx continuously ($\bar{x}$ $f_G = 69{\cdot}3\,min^{-1}$), although the f_G varied considerably among individuals (S.D. = $21{\cdot}8\,min^{-1}$).

As the aquatic P_{O_2} was reduced, the predominance of aerial and aquatic uptake of

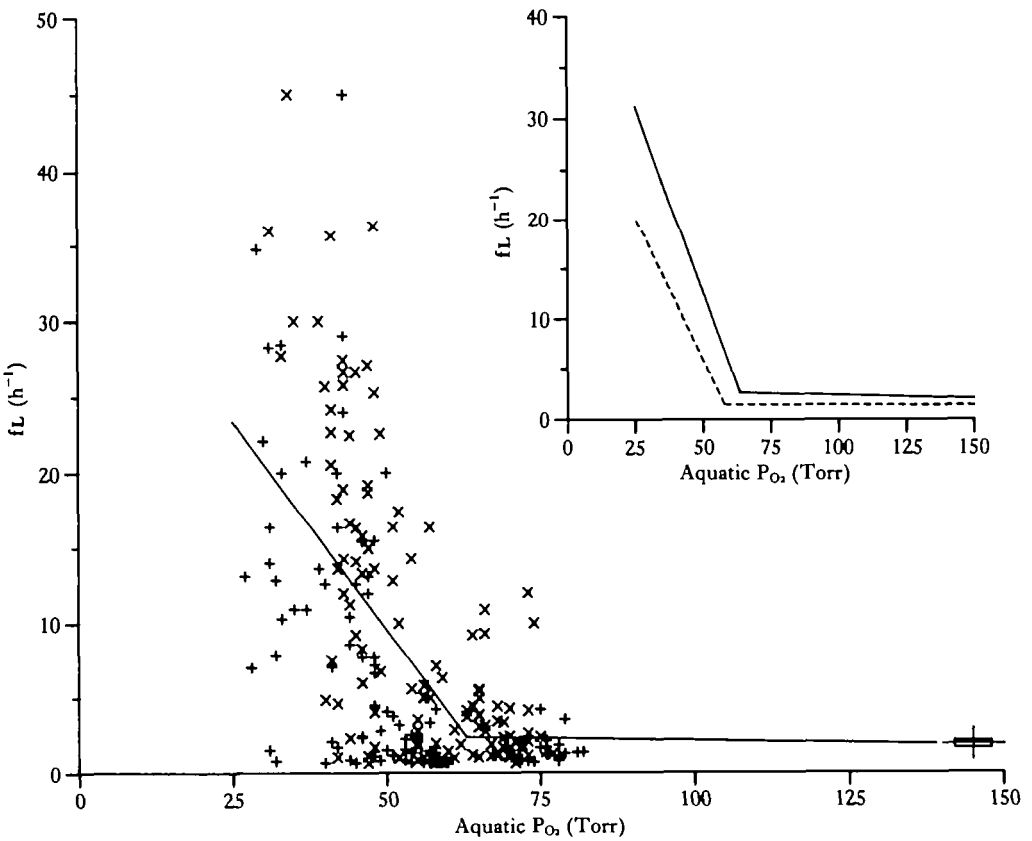

Fig. 1. Effect of aquatic P_{O_2} on lung ventilatory frequency (f_L) in *Xenopus* larvae ($N = 290$). At bottom right, the mean f_L (horizontal line) ± the standard deviation (vertical line) and the 95% confidence interval (open rectangle) are plotted for 77 larvae in normoxic water. Two line segments fitted to all data are also shown. + = larvae <10 mg dry mass, × = larvae >10 mg dry mass. Inset: Line segments fitted to the same data but for larvae <10 mg dry mass (broken line) and larvae >10 mg mass (solid line) separately. The ascending line segments for small and large larvae differ significantly in intercept ($P < 0{\cdot}0001$; analysis of covariance) but not in slope ($P = 0{\cdot}26$).

oxygen reversed (Fig. 2). The aquatic $\dot{V}_{O_2}$ declined dramatically in hypoxia. In fact, below 50 Torr many larvae lost oxygen to the water. A marked increase in the aerial $\dot{V}_{O_2}$ accompanied this reduction in aquatic $\dot{V}_{O_2}$. The increased aerial $\dot{V}_{O_2}$ was insufficient to compensate entirely for the reduced aquatic $\dot{V}_{O_2}$, and the total $\dot{V}_{O_2}$ declined in proportion to the aquatic P_{O_2} below approximately 85 Torr. However, the total $\dot{V}_{O_2}$ was always positive despite the loss of oxygen to the water.

The body size of larvae had little effect upon the proportional changes in $\dot{V}_{O_2}$ in aquatic hypoxia. Of course, large larvae had greater $\dot{V}_{O_2}$ values than small larvae. However, the aquatic $\dot{V}_{O_2}$ and the total $\dot{V}_{O_2}$ of both large and small larvae declined by the same proportion in aquatic hypoxia; both large and small larvae were able to increase aerial $\dot{V}_{O_2}$ in similar proportions to compensate for these declines. These trends were quantified by multiple regression of $\dot{V}_{O_2}$ against aquatic P_{O_2} and body size. The points were fitted to polynomial curves, hyperbolic curves and two line segments (Feder, 1983*a*). Although aquatic P_{O_2} accounted for 45–82% of the variation in aerial, aquatic and total $\dot{V}_{O_2}$, body mass or the interaction of body mass and aquatic P_{O_2} never accounted for more than 3% of the variation in $\dot{V}_{O_2}$, and frequently accounted for much less. The developmental stage of the larvae had little additional

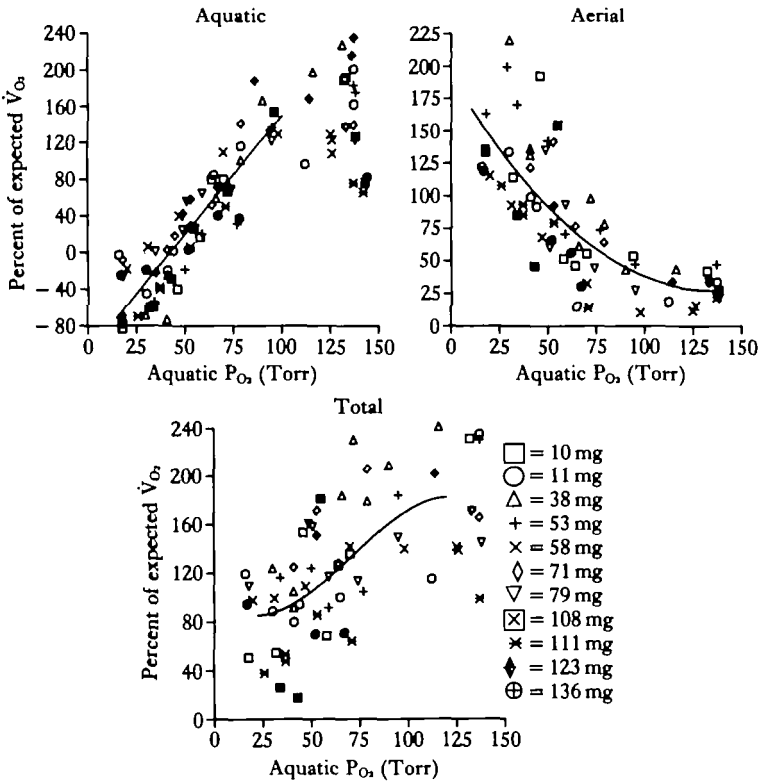

Fig. 2. Effect of aquatic P_{O_2} on aquatic, aerial and total $\dot{V}_{O_2}$. Before plotting, values of $\dot{V}_{O_2}$ (in $\mu l\,h^{-1}$) were transformed by dividing by $2{\cdot}9\,M^{0{\cdot}862}$, which is the routine $\dot{V}_{O_2}$ expected for *Xenopus* larvae of body mass M (mg dry mass) (Feder, 1982). Linear regression of aquatic $\dot{V}_{O_2}$ at $P_{O_2} \leq 100$ yielded the equation: $\dot{V}_{O_2}(\%) = 2{\cdot}6\,P_{O_2} - 110{\cdot}5$ ($r = 0{\cdot}89$). Approximate curves for aerial $\dot{V}_{O_2}$ and total $\dot{V}_{O_2}$ were calculated with polynomial regression, and are plotted only to emphasize trends in the data.

effect upon their $\dot{V}_{O_2}$, but very early and late developmental stages were intentionally excluded from these experiments.

The frequency of air-breathing in aquatic hypoxia corresponded to the change in aerial $\dot{V}_{O_2}$; however, body size affected the fL more markedly than it did the aerial $\dot{V}_{O_2}$. Larvae responded to aquatic hypoxia by increasing the fL dramatically, in some cases up to 24 times the average fL for larvae in normoxic water (Fig. 1). This increase was positively correlated with the size of larvae. At low aquatic P_{O_2}, large tadpoles had a greater fL value than did small tadpoles. Also, large tadpoles began to increase their fL at a greater P_{O_2} than did small tadpoles. Aquatic P_{O_2} and the size of larvae together accounted for 40% of the variance in fL in the sample under observation.

In contrast to the fL, the fG was relatively unaffected by aquatic hypoxia. For the same larvae used for measurements of the fL, the fG was recorded and analysed with multiple regression. Developmental stage, aquatic P_{O_2} and morphological measurements (length, mass, width) statistically accounted for only 7, 4 and 2% of the variance in fG, respectively; 87% of the variance was unexplained. Because the absence of a clear effect of aquatic P_{O_2} on fG might be due to extreme variation among individual larvae, each of six larvae was observed repeatedly for fG and fH as the aquatic P_{O_2} was altered (Fig. 3). Although aquatic P_{O_2} had a near-significant effect on the fG of these larvae ($P = 0{\cdot}077$, Friedman's analysis of variance), the data overall nonetheless reveal no clear change in fG with increasing hypoxia. The fH of these larvae, by contrast, increased somewhat in aquatic hypoxia ($P = 0{\cdot}048$, Friedman's analysis of variance), although the magnitude of this change was also small.

Although the data on buccal floor movement clearly document that *Xenopus* larvae can modify gill stroke volume greatly, there is little evidence that these larvae vary this volume in response to aquatic hypoxia (Fig. 4). The overall correlation coefficient for buccal floor depression and aquatic P_{O_2} was 0·011. Most individual larvae showed significant variation in buccal floor depression as aquatic P_{O_2} was lowered; however,

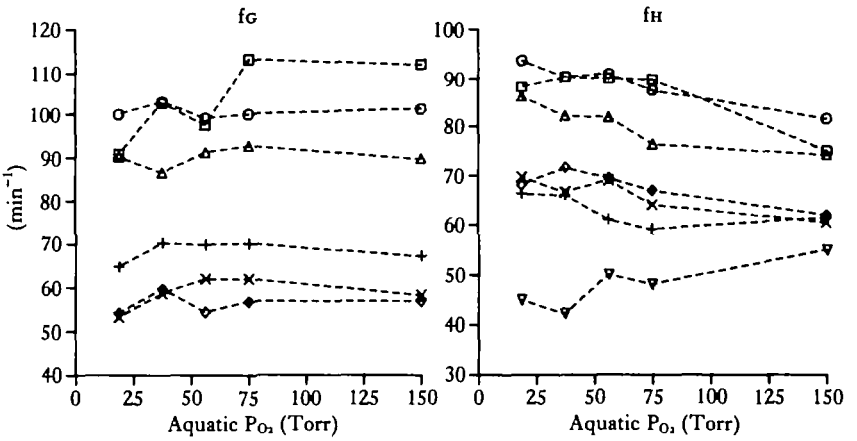

Fig. 3. Buccopharyngeal ventilatory frequency (fG) and heart rate (fH) in individual larvae. These data demonstrate that the fG and the fH are relatively consistent for individual larvae, although variation among individuals may be large. Each tadpole observed is represented by a different symbol. The P_{O_2} significantly affected the fH ($P = 0{\cdot}048$; Friedman's analysis of variance) but not the fG ($P = 0{\cdot}077$), although variation due to the P_{O_2} was not large in either case.

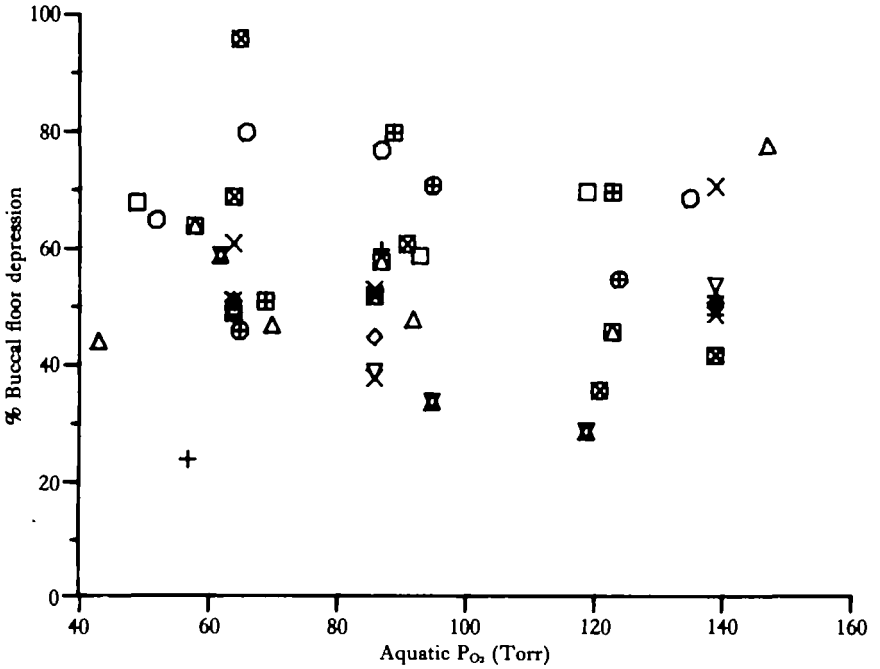

Fig. 4. Effect of aquatic P_{O_2} on the depression of the floor of the mouth during buccopharyngeal ventilation, an indirect measure of buccal stroke volume. The value plotted is calculated from the distance between the top of the head to the buccal floor when the buccal floor was maximally depressed (A) and fully elevated (B) as $100\% \times (A-B)/B$. This value showed little relation to the aquatic P_{O_2} ($R^2 = 0{\cdot}0001$). Each point represents the mean for 5–10 buccal pump strokes. Each tadpole ($N = 15$) is represented by a different symbol.

increases and decreases in buccal floor depression occurred at approximately equal frequencies.

Experimental larvae accumulated little lactate during 1 h exposure to aquatic hypoxia (Table 1). These larvae underwent no physical disturbance before exposure to hypoxia. A second group of tadpoles that were transferred from normoxic water to hypoxic water (and consequently disturbed) showed a 175 % increase in whole body lactate. However, by 4 h after the transfer, lactate concentrations of the tadpoles in hypoxic water had declined to levels only slightly above those for undisturbed tadpoles exposed to aquatic hypoxia for 1 h. These data suggest that production of lactate is not a major response to aquatic hypoxia in *Xenopus* larvae with access to air.

Larvae without access to air

Xenopus larvae without access to air survived indefinitely except at very low aquatic P_{O_2} (see below). For example, six animals without access to air survived for 2 weeks at P_{O_2} between 100–150 Torr and grew during this period.

Exclusively water-breathing *Xenopus* larvae resembled larvae with access to air in their total $\dot{V}_{O_2}$ in normoxic water, but differed from air-breathing larvae in their responses to aquatic hypoxia (Figs 5, 6). One major difference was that exclusively water-breathing larvae did not lose O_2 to the water at low P_{O_2} (i.e. there are no

negative values in Fig. 5A). A second difference was that small and large tadpoles differed in their $\dot{V}_{O_2}$ (relative to $\dot{V}_{O_2}$ expected for resting, air-breathing larvae of the same mass) when denied access to air. This is best seen in Fig. 5B. Unlike air-

Table 1. *Effect of aquatic P_{O_2} on the whole-body lactate concentration in* Xenopus laevis *larvae*

Treatment: Aquatic P_{O_2} (Torr)	Lactate concentration (mg g^{-1} dry mass)
Air-breathing larvae:	
Exposed to indicated P_{O_2} for 1 h without physical transfer:	
150	1·19 ± 0·15 (5)
75	1·78 ± 0·36 (5)
59	2·08 ± 0·56 (5)
38	2·03 ± 0·37 (5)
19	1·32 ± 0·14 (5)
Transferred to indicated P_{O_2} for 4 h:	
150 (analysed immediately after transfer)	3·31 ± 0·43 (6)
96	1·39 ± 0·51 (6)
84	2·43 ± 0·49 (6)
66	2·17 ± 0·33 (6)
34	1·72 ± 0·17 (6)
Exclusively water-breathing larvae:	
150 (4 h)	2·42 ± 0·41 (6)
75 (20 min)	4·28 ± 0·29 (6)
75 (40 min) + 58 (20 min)	4·27 ± 0·58 (6)
75 (40 min) + 58 (40 min) + 38 (20 min)	5·91 ± 1·55 (6)

The lactate concentration is given in mg lactate g^{-1} predicted dry mass (Feder, 1981), and is given as the mean ± S.E. Sample size is given in parentheses.

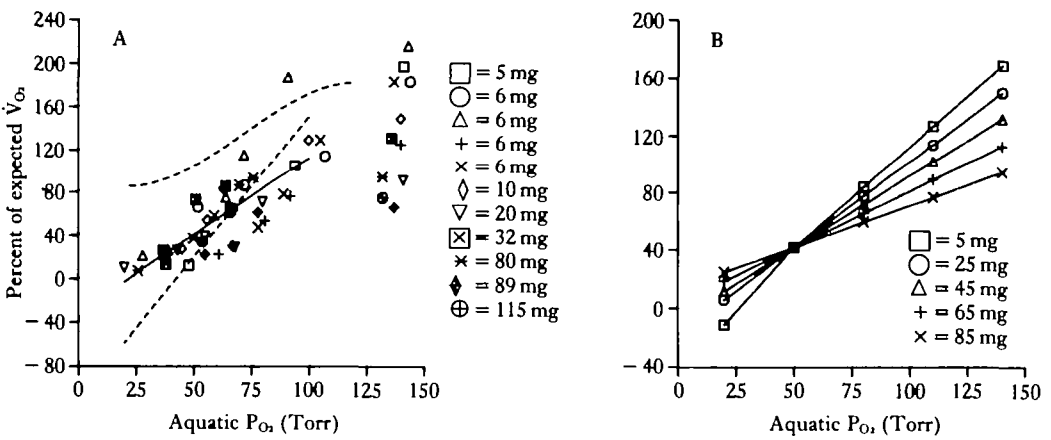

Fig. 5. Effect of aquatic P_{O_2} on total $\dot{V}_{O_2}$ in exclusively water-breathing larvae. The data are scaled as in Fig. 2. (A) Values for 11 individuals. The regression of $\dot{V}_{O_2}$ against P_{O_2} for $P_{O_2} \leq 100$ Torr (solid line) is: $\dot{V}_{O_2}$ (%) = 1·4 P_{O_2} − 31·5 ($r = 0{\cdot}74$). The dashed line is a similar regression for air-breathing larvae (see Fig. 2); the two lines differ significantly in slope ($P < 0{\cdot}001$; analysis of covariance). The dashed curve represents total $\dot{V}_{O_2}$ of air-breathing larvae, and is re-plotted from Fig. 2. (B) Effect of body size of exclusively water-breathing larvae on their $\dot{V}_{O_2}$. The data in (A) were analysed with multiple regression, yielding the equation: $\dot{V}_{O_2}$ (%) = 1·5 P_{O_2} + 0·5 Mass (mg) − 0·01 × P_{O_2} × Mass − 31·4 ($R^2 = 0{\cdot}76$). Representative values of body mass were substituted into this equation to yield the plotted values and lines.

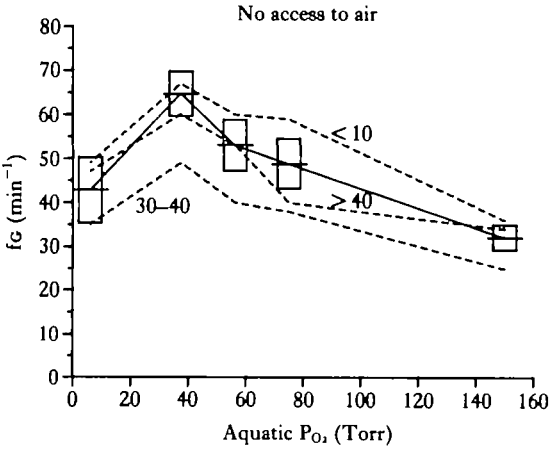

Fig. 6. Effect of P_{O_2} on the fG (buccopharyngeal ventilatory frequency) of exclusively water-breathing larvae. Data are for 80 larvae (mean = 20·5 mg; range = 2–109 mg) observed at each of several P_{O_2} values. Horizontal lines signify mean fG; open rectangles indicate ±95 % confidence intervals. Body size affected the fG of larvae. The solid line connects the mean fG for all larvae. Dashed lines connect mean fG calculated for larvae of three size classes: <10 mg, 30–40 mg, and >40 mg dry mass.

breathing larvae, large tadpoles had $\dot{V}_{O_2}$ values lower than or the same as expected rates, and small tadpoles had $\dot{V}_{O_2}$ values greater than expected rates. Exclusively water-breathing larvae of all sizes were equally successful in minimizing the loss of O_2 to the water in severe hypoxia.

Water-breathing larvae altered their fG in response to aquatic hypoxia. Unlike air-breathing larvae, in which the fG was high and variable at all aquatic P_{O_2} values, the fG of exclusively water-breathing larvae was relatively low at normoxic P_{O_2}, increased markedly at intermediate P_{O_2}, and decreased in severe hypoxia (Fig. 6). This pattern was more marked in large larvae than in small larvae (Fig. 6). Altogether the aquatic P_{O_2} and the body size of larvae accounted for 35–40 % of the variation in fG in various multiple regressions.

Exclusively water-breathing larvae accumulated copious amounts of lactate, both in response to prolonged lack of access to air and relatively brief exposure to hypoxia (Table 1). When exclusively aquatic larvae were exposed sequentially to aquatic P_{O_2} values of 75, 58 and 38 Torr, their lactate concentrations were much greater than in air-breathing larvae that had been exposed to similar intensities of hypoxia for 1–4 h. In a second group of larvae held overnight in normoxic water without access to air, lactate concentrations increased considerably above resting levels, but only in large individuals. The final lactate concentration was positively correlated with body mass (Spearman's $r = 0{\cdot}899$; $P < 0{\cdot}01$).

Consequences of air-breathing

Although *Xenopus* larvae need not breathe air to meet oxygen requirements in normoxia, air-breathing may be necessary for tadpole survival in aquatic hypoxia. During experimentation, air-breathing larvae were often exposed to aquatic P_{O_2} values of 20 Torr or less; mortality was low and occurred only after lengthy exposure

to aquatic hypoxia. For example, six tadpoles survived 11 days at aquatic P_{O_2} between 11–26 Torr. Four survived an additional 2 days, at which time the experiment was discontinued. By contrast, exclusively water-breathing larvae reached the Critical Activity Point at aquatic P_{O_2} between 25–60 Torr (Fig. 7). For 10 such larvae exposed to progressively decreasing aquatic P_{O_2} values, the time until the Critical Activity Point was inversely proportional to their body size (Spearman's $r = -0{\cdot}870$; $P < 0{\cdot}01$).

Air-breathing was also essential to the maintenance of the tadpoles' normal buoyancy and hovering posture (see Introduction). When larvae were placed in

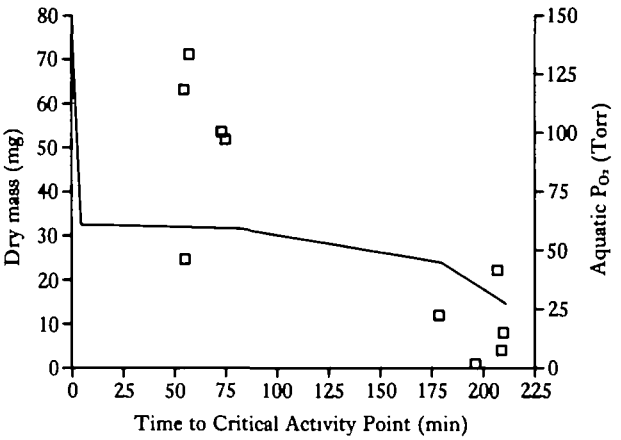

Fig. 7. Tolerance of hypoxia in exclusively water-breathing larvae. The P_{O_2} was reduced according to the schedule represented by the solid line (see scale to right of figure). The times until each of 10 larvae reached the Critical Activity Point are plotted as open squares, and were inversely related to their body size (Spearman's $r = -0{\cdot}870$; $P < 0{\cdot}01$).

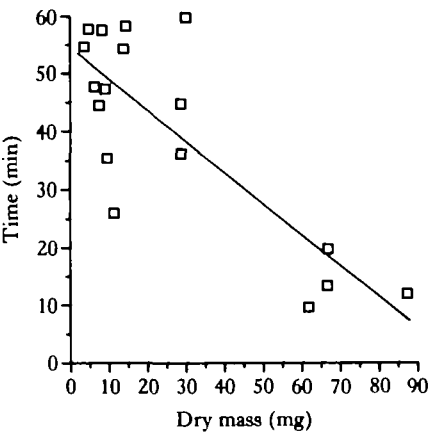

Fig. 8. Time from previous air breath until loss of buoyancy in larvae of different sizes. Larvae were in normoxic water. Loss of buoyancy was identified by the loss of the normal 'head-down' hovering position of larvae. Size and time to loss of buoyancy were inversely related ($r = -0{\cdot}810$; $P < 0{\cdot}01$); results of a linear regression are shown.

containers from which air was excluded, they soon showed difficulties in maintaining this normal posture. Eventually they became negatively buoyant, never hovered stationary in the water column, and only remained midwater by swimming continuously around their containers. Most individuals sank to the bottom and remained there. The time until these tadpoles became negatively buoyant was between 10 and 63 min (Fig. 8), and was inversely related to body size ($r = -0{\cdot}810$). When these animals were again given access to the surface, they regained the normal posture immediately after breathing air. Some tadpoles that had been denied access to the surface and lay on the bottoms of their containers were placed in a pressure chamber and the pressure reduced to −760 Torr. These tadpoles remained on the bottom except when swimming, indicating that their lungs were completely or nearly completely deflated. A group of air-breathing larvae underwent the same treatment. They, however, rose to the water's surface and remained there; they could only descend by vigorous swimming against their excessive buoyancy. Within several minutes these latter tadpoles expired the extra gas as a bubble and thereafter resumed the normal hovering posture.

DISCUSSION

Tadpoles of most anurans have well-developed lungs and breathe air regularly (Savage, 1962; Wassersug & Seibert, 1975; Burggren & West, 1982; Feder, 1983*a*). Aerial respiration is particularly important to tadpoles of *Xenopus*. Their aquatic gas exchangers either may account for little O_2 uptake in aquatic hypoxia or may in fact exacerbate the effects of hypoxia. If, in hypoxia, the environmental P_{O_2} is lower than the P_{O_2} of blood within the gas exchangers, O_2 may be lost to the environment. The features of the gills and skin (large surface area, minimal diffusion barrier) that normally promote O_2 uptake will instead promote O_2 loss. Air breathing compensates for this O_2 loss (Figs 1, 2), and minimizes dependence upon anaerobiosis (Table 1).

Branchial O_2 uptake has not been partitioned from other aquatic routes of gas exchange in the present study. In tadpoles of ranid frogs, which have gill filaments (Savage, 1952, 1962), the gills and other buccopharyngeal surfaces account for no more than 10–40 % of total O_2 uptake (Burggren & West, 1982; West & Burggren, 1982; Burggren, Feder & Pinder, 1983). In *Xenopus* larvae, which lack true gill filaments, this proportion is presumably even less. Inasmuch as *Xenopus* tadpoles with access to air vary neither the f_G nor the buccal pump stroke volume in response to hypoxia (Figs 3–5), it would seem that the buccopharyngeal surfaces of *Xenopus* play but a limited role in respiratory exchange and function primarily in feeding. Indeed, when concentrated food suspensions are added to normoxic water in which exclusively water-breathing *Xenopus* larvae reside, the larvae actually decrease the f_G (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation). This is their normal response to concentrated food suspensions but would presumably decrease aquatic O_2 uptake. Larvae also decrease the f_G during swimming (Wassersug & Feder, 1983), when O_2 demand is elevated.

The buccopharyngeal lining of *Xenopus* tadpoles bears many minute folds, which together with the batteries of branching gill filters have a relatively large surface area (Gradwell, 1971, 1975; Seale, Hoff & Wassersug, 1982). Although these surfaces may

seldom be exploited fully for gas exchange (see above), they can play a significant role in respiration as shown by the increasing and then decreasing f_G when exclusively water-breathing larvae are exposed to increasingly hypoxic water (Fig. 6). In hypoxia, exclusively water-breathing larvae may favour using the buccopharyngeal surfaces for respiration rather than feeding, for the feeding rate declines precipitously in such larvae (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation). Thus, the buccopharyngeal surfaces are accessory respiratory organs that are seldom called upon in normal circumstances.

The skin accounts for the majority of respiratory capillarization (Strawinski, 1956; Saint-Aubain, 1982), is thin, and is the predominant route of O_2 uptake in air-breathing tadpoles in normoxic water (Burggren & West, 1982; West & Burggren, 1982; Burggren *et al.* 1983). Moreover, even though cutaneous gas exchange in amphibians is diffusion-limited along any given capillary (Piiper, 1982), tadpoles can augment cutaneous exchange by perfusing additional capillaries or by increasing cutaneous capillarization and thinning the skin (Burggren & Pinder, 1982). However, these same features enhance the loss of O_2 from air-breathing tadpoles to the water in hypoxia. As in *Xenopus*, tadpoles of *Rana berlandieri* and *Rana catesbeiana* lose O_2 to hypoxic water (West & Burggren, 1982; Feder, 1983*a*).

Although O_2 is lost that could otherwise be utilized by the tissues, this loss is probably inescapable and may not be pernicious. Although tadpoles and adult amphibians can increase cutaneous perfusion greatly (Poczopko, 1957, 1959; Moalli, Meyers, Jackson & Millard, 1980; Burggren *et al.* 1983), they are obviously unable to curtail it entirely to prevent oxygen loss. However, this O_2 loss may well be limited by the O_2 affinity of the blood. The blood O_2 affinity of *Xenopus* tadpoles has not been measured, but the blood of adults is fully saturated above 65 Torr and has a P_{50} of approximately 22 Torr (Emilio & Shelton, 1974). *Xenopus* undergoes an ontogenetic change in haemoglobins; by inference from the situation in *Rana catesbeiana*, this suggests a greater O_2 affinity in larval *Xenopus* than in adults (Broyles, 1981). With such a low P_{50}, only limited deoxygenation of haemoglobin would occur in that portion of the cardiac output distributed to the skin, with the remainder distributed to other tissues. As long as the cardiac output could be increased to offset the partial O_2 loss, adequate tissue oxygenation would be assured. If this scenario is correct, then the increase in cardiac output is primarily *via* increased cardiac stroke volume, for the f_H varies relatively little. Given a high haemoglobin affinity for O_2, *Xenopus* larvae should be able to extract O_2 from all but severely hypoxic water.

The aquatic gas exchangers by themselves can supply 100 % of the routine oxygen requirement in *Xenopus* larvae (Fig. 5). Even so, these larvae still extract 16·6 % or more of required O_2 from the air when in normoxic water. A similar pattern is evident in *Rana* larvae (Feder, 1983*a*). This continued reliance on aerial oxygen, while unnecessary by strictly respiratory considerations, may be related to several factors in *Xenopus*.

First, as noted above, the aquatic gas exchangers alone are inadequate in responding to aquatic hypoxia. Regular air-breathing, which would maintain a store of O_2 in the lungs, may prepare tadpoles for encounters with hypoxia (Emilio & Shelton, 1974; Randall, Burggren, Farrell & Haswell, 1981). The P_{O_2} of most tadpole habitats is unknown. However, Savage (1952, 1962) and Noland & Ultsch (1981) have

documented substantial spatial and temporal variation in the P_{O_2} of a few typical tadpole habitats. Habitats of *Xenopus* tadpoles range from permanently flowing water to temporary ponds to water buffalo wallows, and thus larvae may routinely encounter aquatic hypoxia.

A second reason for routine air-breathing in *Xenopus* may lie in its linkage to normal feeding and hovering behaviour. Prevention of air-breathing leads to rapid loss of the normal hovering posture (Fig. 8), and possibly loss of audition, proprioception and balance as well (van Bergeijk, 1959; Gradwell, 1971). While negatively buoyant tadpoles can survive in the laboratory, they may not be so fortunate in the wild. *Xenopus* larvae are unusual in that they have a large oral orifice and lack the cornified structures and oral papillae that guard this opening in most tadpoles. As such, a *Xenopus* tadpole on the bottom of a pond risks clogging its gill filters with silt and detritus. In hypoxic water, prevention of air-breathing leads to a decrease in the rate of food ingestion (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation) and accumulation of lactate (Table 1). Accordingly, it is advantageous for *Xenopus* larvae to breathe air regularly. It is not known whether *Xenopus* larvae or other buoyant tadpoles alter lung volume to regulate buoyancy in the manner of some fish (Gee & Gee, 1976). The positive buoyancy resulting from the lungs, however, reduces stamina during sustained swimming (Wassersug & Feder, 1983).

A welcome trend in recent studies of air-breathing in aquatic vertebrates is the analysis of respiratory responses in the context of the many other activities that organisms must carry on: osmoregulation, locomotion, predator avoidance, feeding, etc. (e.g. Randall *et al.* 1981; Kramer, 1983; Lauder, 1983). Such an approach is obligatory in understanding the respiratory responses of tadpoles, in which the exchange of gasses, feeding (M. E. Feder, D. B. Seale, M. E. Boraas, R. J. Wassersug & A. G. Gibbs, in preparation), locomotion (Wassersug & Feder, 1983), and osmoregulation (Dietz & Alvarado, 1974) either are intimately related or are performed by the same structures. From a respiratory perspective alone, many responses of *Xenopus* (e.g. the failure to increase f_G in hypoxia, the loss of O_2 to the water) seem bizarre. However, these responses may be innocuous in terms of a tadpole's growth and development.

We thank R. Paul, T. Holtsford, A. Gibbs, L. Johnson and N. Ronczy for technical assistance. A semi-anonymous referee provided helpful comments. Research was supported by NSF Grant DEB 78-23896, NSERC, and The Louis Block Fund, The University of Chicago.

REFERENCES

Bles, E. J. (1905). The life history of *Xenopus laevis* Daudin. *Trans. R. Soc. Edinb.* **41**, 789–821.

Broyles, R. H. (1981). Changes in the blood during amphibian metamorphosis. In *Metamorphosis, a Problem in Developmental Biology*, 2nd ed., (eds L. I. Gilbert & E. Frieden), pp. 461–490. New York and London: Plenum Press.

Burggren, W. W., Feder, M. E. & Pinder, A. W. (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* **56**, 263–273.

BURGGREN, W. W. & PINDER, A. W. (1982). Chronic hypoxia produces different hematological and respiratory morphometric effects in larval vs. adult bullfrogs (*Rana catesbeiana*). *Physiologist* **25**, 249.

BURGGREN, W. W. & WEST, N. H. (1982). Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog, *Rana catesbeiana*. *Respir. Physiol.* **47**, 151–164.

DIETZ, T. H. & ALVARADO, R. H. (1974). Na and Cl transport across gill chamber epithelium of *Rana catesbeiana* tadpoles. *Am. J. Physiol.* **226**, 764–770.

EMILIO, M. G. & SHELTON, G. (1974). Gas exchange and its effect on blood gas concentrations in the amphibian, *Xenopus laevis*. *J. exp. Biol.* **60**, 567–579.

FEDER, M. E. (1981). Effect of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. *Comp. Biochem. Physiol.* **70**A, 497–508.

FEDER, M. E. (1982). Effect of body size and developmental stage on oxygen consumption of anuran larvae: a reappraisal. *J. exp. Zool.* **220**, 33–42.

FEDER, M. E. (1983*a*). Responses to acute aquatic hypoxia in larvae of the frog *Rana berlandieri*. *J. exp. Biol.* **104**, 79–95.

FEDER, M. E. (1983*b*). The relation of air-breathing and locomotion to predation on tadpoles (*Rana berlandieri*) by turtles. *Physiol. Zool.* **56**, (in press).

GEE, J. H. (1968). Adjustment of buoyancy by longnose dace (*Rhinichthys cataractae*) in relation to velocity of water. *J. Fish. Res. Bd Can.* **25**, 1485–1496.

GEE, J. H. & GEE, P. A. (1976). Alteration of buoyancy by some Central American stream fishes, and a comparison with North American species. *Can. J. Zool.* **54**, 386–391.

GOSNER, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.

GRADWELL, N. (1971). *Xenopus* tadpole: on the water pumping mechanism. *Herpetologica* **27**, 107–123.

GRADWELL, N. (1975). The bearing of filter feeding on the water pumping mechanism of *Xenopus* tadpoles (Anura: Pipidae). *Acta zool., Stockh.* **56**, 119–128.

KRAMER, D. L. (1983). The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. *Env. Biol. Fishes* (in press).

KRAMER, D. L., MANLEY, D. & BURGEOIS, R. (1983). The risk of respiration in fishes. *Can. J. Zool.* **61**, 653–665.

LAUDER, G. V. (1983). Prey capture hydrodynamics in fishes: experimental tests of two models. *J. exp. Biol.* **104**, 1–13.

MOALLI, R., MEYERS, R. S., JACKSON, D. C. & MILLARD, R. W. (1980). Skin circulation of the frog, *Rana catesbeiana*, distribution and dynamics. *Respir. Physiol.* **40**, 137–148.

NOLAND, R. & ULTSCH, G. R. (1981). The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). *Copeia* **1981**, 645–652.

PIIPER, J. (1982). A model for evaluating diffusion limitation in gas-exchange organs of vertebrates. In *A Companion to Animal Physiology*, (eds C. R. Taylor, K. Johansen & L. Bolis), pp. 49–64. Cambridge: Cambridge University Press.

POCZOPKO, P. (1957). Further investigations on the cutaneous vasomotor reflexes in the edible frog in connexion with the problem of regulation of the cutaneous respiration in frogs. *Zoologica Pol.* **8**, 161–175.

POCZOPKO, P. (1959). Changes in blood circulation in *Rana esculenta* while diving. *Zoologica Pol.* **10**, 29–43.

RANDALL, D. J., BURGGREN, W. W., FARRELL, A. P. & HASWELL, M. S. (1981). *The Evolution of Air Breathing in Vertebrates*. Cambridge: Cambridge University Press.

SAINT-AUBAIN, M. L. (1982). The morphology of amphibian skin before and after metamorphosis. *Zoomorphology* **100**, 55–63.

SAVAGE, R. M. (1952). Ecological, physiological and anatomical observations on some species of anuran tadpoles. *Proc. zool. Soc. Lond.* **122**, 467–514.

SAVAGE, R. M. (1962). *The Ecology and Life History of the Common Frog*. New York: Hafner Publishing Co.

SCHOLANDER, P. F., CLAFF, C. L., TENG, C. T. & WALTERS, V. (1951). Nitrogen tension in the swimbladder of marine fishes in relation to the depth. *Biol. Bull. mar. biol. Lab., Woods Hole* **101**, 178–193.

SEALE, D. B., HOFF, K. & WASSERSUG, R. (1982). *Xenopus laevis* larvae (Amphibia, Anura) as model suspension feeders. *Hydrobiologia* **87**, 161–169.

SEALE, D. B. & WASSERSUG, R. J. (1979). Suspension feeding dynamics of anuran larvae related to their functional morphology. *Oecologia* **39**, 259–272.

STRAWINSKI, S. (1956). Vascularization of respiratory surfaces in ontogeny of the edible frog, *Rana esulenta* [sic] L. *Zoologica Pol.* **7**, 327–365.

VAN BERGEIJK, W. A. (1959). Hydrostatic balancing mechanism of *Xenopus* larvae. *J. acoust. Soc. Am.* **31**, 1340–1347.

WASSERSUG, R. J. (1972). The mechanism of ultraplanktonic entrapment in anuran larvae. *J. Morph.* **132**, 279–288.

WASSERSUG, R. J. & FEDER, M. E. (1983). The effects of aquatic oxygen concentration, body size, and

respiratory behaviour on the stamina of obligate aquatic (*Bufo americanus*) and facultative air-breathing (*Xenopus laevis* and *Rana berlandieri*) anuran larvae. *J. exp. Biol.* **105**, 173–190.

WASSERSUG, R. J. & SEIBERT, E. A. (1975). Behavioral responses of amphibian larvae to variation in dissolved oxygen. *Copeia* **1975**, 86–103.

WEST, N. H. & BURGGREN, W. W. (1982). Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole (*Rana catesbeiana*). *Respir. Physiol.* **47**, 165–176.